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Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line from a patient with Leber congenital amaurosis

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A B S T R A C T

Leber congenital amaurosis (LCA) is an inherited retinal dystrophy that is characterized by severe visual impairment in early infancy. We generated a human induced pluripotent stem cell (hiPSC) line, DKHi090-A, from peripheral blood mononuclear cells (PBMCs) of a patient with LCA, by using a Sendai virus-based gene delivery system. We confirmed that DKHi090-A has a nicotinamide mononucleotide adenyltransferase 1 (NMNAT1) mutation and normal karyotype. DKHi090-A line is pluripotent and is capable of multilineage differentiation. This cell line is registered and is available at the National Stem Cell Bank, Korea National Institute of Health.

Resource table

Unique stem cell line identifier	DKHi090-A
Alternative name(s) of stem cell line	DKH090i-A
Institution	Korea National Institute of Health
Contact information of distributor	Soo Kyung Koo, skkoo@nih.go.kr
Type of cell line	iPSC
Origin	Human
Additional origin info	Applicable for human iPSC Age: 1 year Sex: female Ethnicity if known: Korean
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Transgene free Sendai virus
Genetic Modification	Yes
Type of Modification	Spontaneous mutation
Associated disease	Leber congenital amaurosis
Gene/locus	NM 022787.3(NMNAT1): c.709C>T/Chr1: 9982570(GRCh38)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	June 2018
Cell line repository/bank	Korea National Stem Cell Bank(KSCB)
Ethical approval	Yonsei University Health System, Severance Hospital, Institutional Review Board(IRB) approval obtained (IRB No. 4-2016-1158) IRB approval obtained (IRB No. 2017-03-05-C-A)

1. Resource utility

Leber congenital amaurosis (LCA) is a severe retinal disorder (den Hollander et al., 2008). Various genetic mutations have been reported in patients with the disease. However, the mechanism underlying nicotinamide mononucleotide adenyltransferase 1 (NMNAT1)(Alias: LCA9) mutation-induced LCA is poorly understood. This hiPSC line might serve as an effective disease model to study LCA9 pathogenesis.

2. Resource details

To date, mutations in 26 genes have been reported as the cause of Leber congenital amaurosis (LCA). These genes are mostly involved in the phototransduction cascade in retinal photoreceptor cells. However, mutations in nicotinamide mononucleotide adenyltransferase 1 (NMNAT1) (Alias: LCA9) gene, which is involved in NAD+ biosynthesis, lead to severe macular coloboma in infancy (Siemiatkowska et al., 2014). Although NMNAT1 activity is critical for normal cellular metabolism in most tissues, changes in phenotype have been reported only in the retina. Hence, study of its functional role in the retina is important for understanding the pathogenesis of LCA9 and designing novel drugs for retinal dystrophy. The patient was a 6-month-old girl who was under evaluation in our institution, for poor eye contact. The

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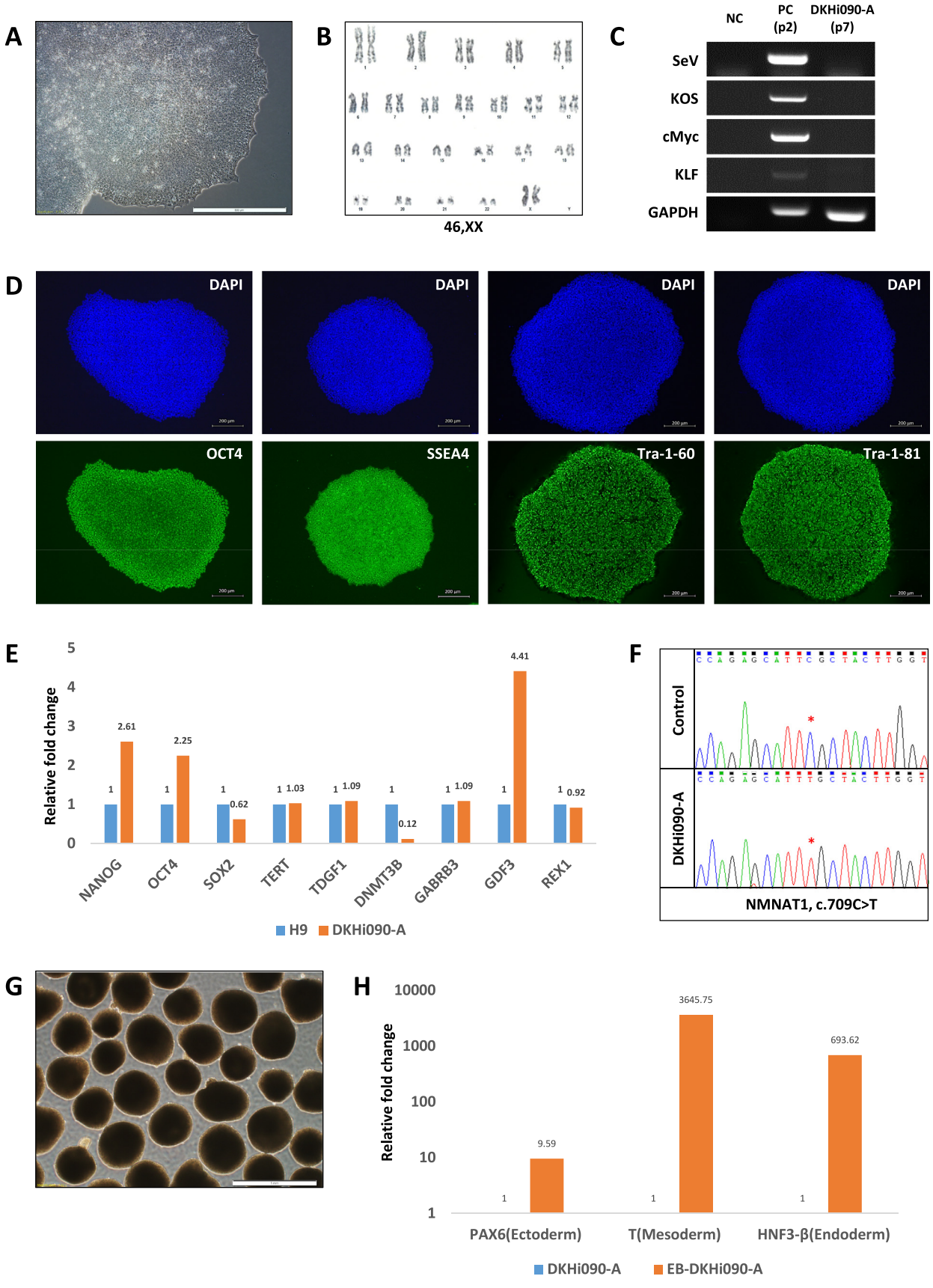

Fig. 1. Characterization of DKHi090-A cell line.

pregnancy and birth were uneventful, and she was born as a normal healthy baby. At the age of 3 months, wandering eye movement was noted, and she was referred to our clinic. The pupil was sluggish, reactive to light, and could not maintain fixation. Dilated fundus examination showed macular coloboma retinal degeneration in both the eyes. Neurological examination was normal. Magnetic resonance imaging for the brain was also normal. Targeted panel sequencing analysis revealed NMNAT1 c.709C > T:p.Arg237Cys homozygous mutation. This variant was previously reported in LCA patients. We generated the hiPSC line, DKHi090-A, by reprogramming the PBMCs of the LCA patient under study by using the Sendai virus-based gene delivery system. The generated line showed a typical hiPSC morphology with a large nuclear to cytoplasmic ratio (Fig. 1A). Moreover, our G-banding analysis showed a normal karyotype (46, XX) in these cells (Fig. 1B). All reprogramming factors were found to be silenced in the DKHi090-A hiPSC line (Fig. 1C). The pluripotency of the generated hiPSC line was characterized by immunocytochemistry analysis using pluripotency markers such as OCT4, SSEA4, Tra-1-60, and Tra-1-81 (Fig. 1D). We also confirmed pluripotency at the mRNA level by using TaqMan® expression probes. The results were analyzed based on the embryonic stem cell line, H9 (Fig. 1E). The disease-causing mutation (C709T) was confirmed in the DKHi090-A hiPSC line (Fig. 1F). To determine the differentiation ability, we generated embryoid bodies from the hiPSC line (Fig. 1G). Using Taqman® expression probes, we confirmed that these embryoid bodies expressed all three germ layer markers, PAX6 (ectoderm), T (mesoderm), and HNF3-β (endoderm) (Fig. 1H). Short tandem repeat (STR) analysis was performed on DKHi090-A and the donor cells. Sixteen allele loci of DKHi090-A matched with those of the donor cells. Detailed information on STR analysis is presented in Supplementary data. DKHi090-A was free from mycoplasma contamination (Supplementary Fig. 1) (Table 1).

3. Materials and methods

3.1. Isolation of PBMCs

PBMCs were isolated using SepMate (Stemcell Technologies, 15450), according to the manufacturer's instructions. Briefly, the density gradient medium was added to the SepMate tube, following which the diluted sample was added to the tube. The tube was then centrifuged at 1200 g for 10 min at room temperature. The topmost layer was then poured off into a new tube and washed twice with PBS.

3.2. Reprogramming and hiPSC maintenance

Human iPSCs were generated by reprogramming PBMCs using a CytotuneiPS™-iPS 2.0 Sendai Reprogramming Kit (Life Technologies, A16518), according to the manufacturer's protocol (Fusaki et al., 2009). Obtained clones were cultured onto vitronectin (Gibco, A14700) coated plates in TeSR™-E8™ medium (Stemcell Technologies, 05990) at 37 °C in a 5% CO_2 atmosphere. The culture medium was changed every day. Cells were passaged using 0.5 mM EDTA (Invitrogen, 15575-020) with 10 μM Y-27632 (Sigma, Y-0503).

3.3. Karyotyping

The cultured cells were treated with colcemid for 45 min and then harvested in hypotonic solution with fixative, following which the metaphase slides were prepared. After Giemsa-trypsin banding, the karyotype was analyzed according to the International System for Human Cytogenetic Nomenclature using the standard GTG-banding method.

3.4. Detection of the reprogramming vector

Total RNA was isolated using a Maxwell® RSC simplyRNA Cells Kit (Promega, AS1390), and cDNA was synthesized by reverse transcription using RNA to cDNA EcoDry Premix (Clontech, 639543). hiPSCs at passage 2 were used as a positive control. PCR (C1000 Touch Thermal Cycler, BIO-RAD) was performed as follows: 95 °C for 5 min, 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s (32 cycles), 72 °C for 5 min, and maintained at 4 °C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified concurrently and used as an internal control. A negative control was included in all runs with all of the same components except the template to detect contamination with nucleic acids.

3.5. Mutation sequencing

Genomic DNA was isolated using a Maxwell® RSC Blood DNA Kit (Promega) and sent to Cosmogenetech (Seoul, Korea) with designed primers. The control cell line used here was the KSCBi002-B, which is already registered in the hPSCreg.

3.6. Immunocytochemistry

Cells were fixed using 4% paraformaldehyde (Wako, 163-20145) for 20 min, blocked with 5% bovine serum albumin (Sigma-Aldrich, A9647) with 0.25% Triton X (Sigma-Aldrich, A8787), and incubated with primary antibodies for Oct3/4, SSEA4, Tra-1-60, and Tra-1-81 (Table 2). Images were acquired using a fluorescence microscope (Axio Imager A2, Carl Zeiss). Scale bars represent 200 μm.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	OCT4, SSEA4, Tra-1-60 and Tra-1-81	Fig. 1 panel D
	Real-time PCR	NANOG, OCT4, SOX2, TERT, TDGF1, DNMT3B, GABRB3, GDF3 and REX1	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 500	Fig. 1 panel B
Identity	Microsatellite PCR(mPCR)	Not performed	Not performed
	STR analysis	Tested 16 loci, all matched	STR analysis
Mutation analysis (IF APPLICABLE)	Sequencing	Homozygous	Fig. 1 panel F
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR negative	Supplementary figure 1
Differentiation potential	Embryoid body formation	Paired box 6 (PAX6) for ectoderm, Brachyury (T) for mesoderm and Hepatocyte nuclear factor-3 beta (HNF-3β) for endoderm	Fig. 1 panel G and H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not performed
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

Table 2
Reagents details.
RRID Requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Marker	Rabbit anti-OCT3/4	1:200	Santa Cruz Biotechnology Cat# sc-9081, RRID:AB_2167703
Pluripotency Marker	Mouse anti-SSEA4	1:200	Millipore Cat# MAB4304, RRID:AB_177629
Pluripotency Marker	Mouse anti-TRA-1-60	1:200	Millipore Cat# MAB4360, RRID:AB_2119183
Pluripotency Marker	Mouse anti-TRA-1-81	1:200	Millipore Cat# MAB4381, RRID:AB_177638
Secondary antibody	Anti-rabbit IgG(H + L) Fluorescein conjugated	1:500	Vector Laboratories Cat# FI-1000, RRID:AB_2336197
Secondary antibody	Anti-mouse IgG(H + L) Fluorescein conjugated	1:500	Vector Laboratories Cat# FI-2000, RRID:AB_2336176

Primers		
	Target	Forward/Reverse primer (5′-3′)
Sendai virus test(RT-PCR)	SeV	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
Sendai virus test(RT-PCR)	KOS	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
Sendai virus test(RT-PCR)	Klf4	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA
Sendai virus test(RT-PCR)	c-Myc	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG
Targeted mutation analysis/sequencing	NMNAT1, 709C > T	GTTACTCGGGCTGGAAATGA/ CCCAAATGGGAAGTCTGAAA
House-Keeping Gene(RT-PCR)	GAPDH	CATGTTCGTCATGGGTGTGAA/ GGACTGTGGTCATGAGTCCTT
Pluripotency Marker (qPCR)	NANOG	Hs02387400-g1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	OCT4	Hs00742896-s1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	SOX2	Hs00602736-s1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	TERT	Hs00162669-m1 (Taqman® probe ID)
Pluripotency Marker (qPCR)	TDGF1	Hs02339499-g1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	DNMT3B	Hs00171876-m1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	GABRB3	Hs00241459-m1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	GDF3	Hs00220998-m1 (Taqman® Probe ID)
Pluripotency Marker (qPCR)	REX1	Hs00399279-m1 (Taqman® Probe ID)
Differentiation Marker (qPCR)	PAX6	Hs00240871-m1 (Taqman® Probe ID)
Differentiation Marker (qPCR)	T (Brachyury)	Hs00610080-m1 (Taqman® Probe ID)
Differentiation Marker (qPCR)	HNF-3β	Hs00232764-m1 (Taqman® Probe ID)
House-Keeping Gene (qPCR)	GAPDH	Hs999999905-m1 (Taqman® Probe ID)

3.7. Real-time PCR analysis

Real-time PCR (QuantStudio™6 Flex, Applied Biosystems) was performed using Taqman® Gene Expression Master Mix (Applied Biosystems, 4369510). PCR conditions were as follows: 50 ℃ for 2 min, 95 ℃ for 10 min at the hold stage, and 95 ℃ for 15 s, 60 ℃ for 1 min at the PCR stage for 40 cycles. All quantitative gene expressions were normalized to the expression level of GAPDH. The probes used are listed in Table 2.

3.8. In vitro differentiation into the three germ layers

To determine the capacity of the hiPSCs to differentiate into the three germ layers, we induced embryoid bodies using hiPSCs harvested by dispase (Gibco, 17105-041). The culture medium for embryoid bodies was DMEM/F12 (Gibco, 11320-082) supplemented with 20% Knockout Serum Replacement (Gibco, 10828-028), 0.1 mol/L Minimal Essential Media non-essential amino acids solution (Gibco, 11140-050), 0.1 mmol/L 2-mercaptoethanol (Gibco, 21985-023), 1% (v/v) antibiotic-antimycotic (Gibco, 15240-112), and MycoZap plus-PR (Lonza, VZA2021). The culture medium was changed every other day. The embryoid bodies were harvested after 14 days and real-time PCR analysis was conducted for the three germ layer. The probes used are listed in Table 2.

3.9. STR analysis

STR analysis of the generated hiPSCs and parental cells was performed using a PowerPlex 16 System (Promega) to detect 16 loci at the Samkwang Medical Laboratories (Seoul, Korea).

3.10. Mycoplasma test

The Mycoplasma test of the cell culture medium was performed using a PCR Mycoplasma Detection Set (TAKARA, 6601) according to the manufacturer's protocol. Briefly, we mixed the cell culture medium with the 1st PCR reaction mixture and performed the 1st PCR. The 1st PCR product was added into the 2nd PCR reaction mixture and the 2nd PCR was performed. Gel electrophoresis was performed using the products from the 1st and 2nd PCRs to confirm the presence of mycoplasma.

Declaration of Competing Interest

There are no conflicts of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101725.

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